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Cultural Physiology: Effect of Culture Mediums and pH on the Growth, Sporulation and Secondary Metabolites Production of *Aspergillus Umbrosus*

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Abstract

Selection of a suitable medium, pH, incubation period, temperature etc., for the cultivation of microorganisms are very important for their growth, sporulation and their ability to produce secondary metabolites, for product formation like organic acid, fermented products and antibiotic production etc. The experiment carried out for the objectives include determination of the effects of nutritional and cultural factors in an in-vitro study were performed for *Aspergillus umbrosus*. Several culture mediums were experimented for the studies. In this paper records given for three synthetic culture mediums tested was, Asthana & Hawker's, Pferrer's, and Currie's at different pH viz.; 3.5, 4.0, 4.5, 5.0 & 5.5. The incubation period of 12 days, culture broth medium was taken, temperature 30°C, dry mycelium weight was taken as biomass. Sporulation was visualised, secretion of secondary metabolites was also observed as an exudates, creates reverse colouration in the medium. In all three mediums experimented the growth of *A. umbrosus* was found nil at pH 3.5 in Asthana and Hawker's and Currie's medium, and in all cases it was optimum at pH 5.0 after that at pH 5.5 there were decrease in growth rate, sporulation was moderate & secondary metabolite production in the form of reverse coloration were moderate i.e. light brown in Currie's medium. Results indicated that amongst these three medium tested fungal growth, sporulation & secondary metabolite production is the best in Currie's medium but it was also not up to satisfactory level.

Key Words: *Aspergillus umbrosus*, Culture medium, pH, Growth, Sporulation, Secondary metabolites

Introduction

The concept of cultural medium does not only imply a definite quantitative composition of the medium, which is required for constructive and energy metabolism of the organism, it also implies physicochemical each factor separately plays an important role not only in the growth of microorganisms, but also in the activation of their physiological and biochemical function. As a matter of fact a useful fungus has to be evaluate first for its morphology, physiology and biochemistry; and then in respect of its genetics and genetic improvement. In almost all of the industrial fungi thorough investigations about their growth behaviour, metabolism and pathway in product formation have been understood under cultural conditions. Optimization of the cultural conditions, including the environment factors, is essential in the fermentation industry for optimized production of the desired product. Pitt *et. al.* (1983) investigated an improved medium for the enumeration of *A. flavus* and *Aspergillus paraciticus* a medium was developed by the modification of Bothast and Fennel's *Aspergillus* differential medium. Nieminen *et.al.* (2002) studied about the isolation and identification of *A. fumigatus* mycotoxins on growth medium. Kulshreshta and Ali (1986) have reported that *A. umbrosus* (Bainier & Sartory) have possessed a good antifungal activity and could be exploited as bio-fungicide. They also observed that the fungus was a difficult organism because of very slow rate of growth and minimal sporulation. All this information about *A. umbrosus* evoked much interest in growth behaviour, suitable environmental and physical factors under cultural conditions.

Materials and Methods

A study was undertaken to investigate *A.umbrosus*, with respect to its physiology of growth and antibiotic production, in the form of secondary metabolite production and to evaluate it for some practical application. The objective was to obtain better vegetative growth, good sporulation and good amount of antibiotic production. Lo *et. al.* (1967) studied on the isolation of aflatoxin by growing *Aspergillus flavus* 3734/10 under the controlled conditions on a modified Czapek's medium with rice powder or crushed peanuts; an average of 80 mg of aflatoxin per litre of liquid medium was produced. The ratio of aflatoxin production of the medium with rice powder to that without rice powder was approximately 5 to 1. Agnihotri (1964) investigated the effect of pH on the growth and sporulation of *A. nidulans.* Wint, *A. rugulosus* Thom *et.* Raper and *A. quadrilineatus* and found that all could grow on a wide range of pH (2.0 – 11.0) but the growth was poor on too acidic or too alkaline media. The

best growth of *A. rugulosus, A. quadrilineatus* and *A. violaceus* was at pH 6.5 and that of *A. nidulans* and *A. veriecolor* at pH 7.0, maximum production of perithecia was recorded between pH 6.0 and 8.0. The experiments carried out for the objectives include determination of the effects of nutritional and cultural factors in an in-vitro study were performed for *A. umbrosus*. Several culture medium were experimented, three of them were given here. The culture mediums used for the studies were: - Asthana and Hawker's Medium (Glucose: 5.0 g; KNO_3: 3.5 g; KH_2PO_4: 1.75 g; $MgSO_4.7H_2O$: 0.75 g; Distilled water: 1000 ml.); Pferrer's Medium (Ammonium tartarate: 10.0 g; KH_2PO_4: 5.0 g; $MgSO_4.7H_2O$: 2.5 g, Sucrose: 50.0 g, $FeSO_4.7H_2O$: Trace; Distilled water: 1000 ml.); Currie's Medium (Ammonium nitrate: 2.0 g; KH_2PO_4: 0.75 g; $MgSO_4.7H_2O$: 0.25 g; Sucrose: 125.0 g; Distilled water: 1000 ml.). In all three mediums experiment were performed at 5 different pH viz. 3.5, 4.0, 4.5, 5.0 & 5.5. The pH values were set before autoclaving. In each case the fungus was grown in Broth medium after being inoculated uniformly (a 4 mm disc of freshly grown colony of *A. umbrosus* per 25 ml medium). The incubation period of 12 days, culture broth medium was taken, temperature 30°C, dry mycelium weight was taken as biomass. Sporulation was visualised, secretion of secondary metabolites was also observed as an exudates (pigment production), creates reverse colouration in the medium.

Results

Studies carried out to determine the effects of different culture medium upon the organism *A. umbrosus* have presented some results to elucidate its nutritional physiology related with its growth, sporulation and secondary metabolites production at different pH. Out of three culture mediums experimented result indicated that in Asthana & Hawker's medium at pH 3.5, no growth was visualized, it was minimum at pH 4.5 (43 mg/ 25 ml) & it was maximum at pH 5.0 (69 mg/25 ml.). Degree of sporulation was also very poor & secondary metabolites production observed in the form of reverse colouration was also less. In Pferrer's medium fungal growth was minimum at pH 3.5 (67 mg/25 ml) and maximum at pH 5.0 (139 mg/25 ml) & degree of sporulation was moderate & reverse coloration was also moderate. In Currie's medium at pH 3.5 no growth was visualised, minimum growth was found (29 mg/25 ml.) at pH 4 & the maximum growth was found (170 mg/25 ml.) at pH 5.0, and Secretion of secondary metabolites also mentioned in the form of exudates which were experimented to show as reverse coloration which is dark brown in the broth medium resulted optimum at pH 5.0. In all three mediums were experimented fungal growth were found nil at pH 3.5 in Asthana and Hawker's and Currie's medium, and in all cases it was optimum at pH 5.0, after that at pH 5.5 there were decrease in growth rate, sporulation was moderate & secondary metabolite production in the form of reverse coloration were moderate i.e. light brown in Currie's medium. Results indicated that amongst these three medium tested fungal growth, sporulation & secondary metabolite production was the best in Currie's medium but it was also not up to satisfactory level. (Table and fig.1, 2 and 3)

Table: - Growth & sporulation of *Aspergillus umbrosus* in synthetic medium at different pH

S. No.	Cultural medium	pH of the medium	Growth (Biomass in mg/25 ml)	Degree of sporulation	Reverse coloration
1.	Asthana & Hawker's	3.5	Nil	-	-
		4.0	45	1^+	Greenish yellow
		4.5	43	1^+	Greenish yellow
		5.0	69	1^+	Greenish yellow
		5.5	65	1^+	Greenish yellow
2.	Pferrer's	3.5	67	2^+	Greenish brown
		4.0	120	3^+	Greenish brown
		4.5	128	3^+	Greenish brown
		5.0	139	3^+	Greenish brown
		5.5	131	3^+	Greenish brown
3.	Currie's	3.5	Nil	-	-
		4.0	29	1^+	Light yellow
		4.5	165	3^+	Light brown
		5.0	170	3^+	Light brown
		5.5	162	3^+	Light brown

Degree of sporulation: - 1^+ = Very poor, 2^+ = Poor, 3^+ = Moderate, 4^+ = Good.
Temperature: - 30°C, Incubation period 12 days.

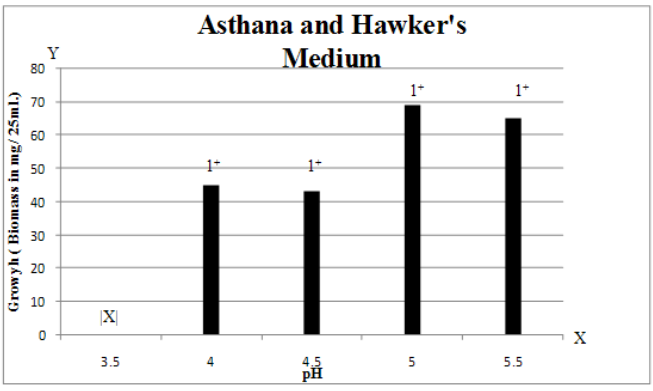

Fig. 1

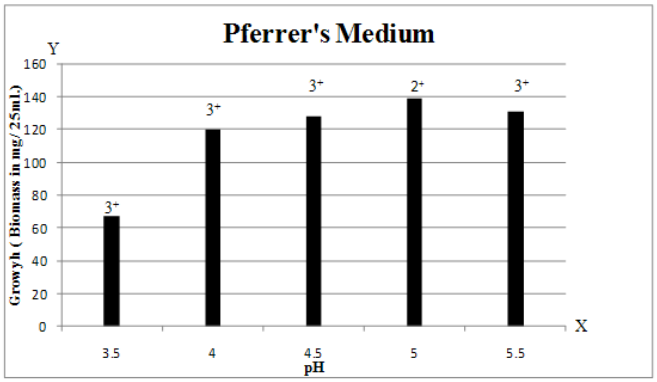

Fig. 2

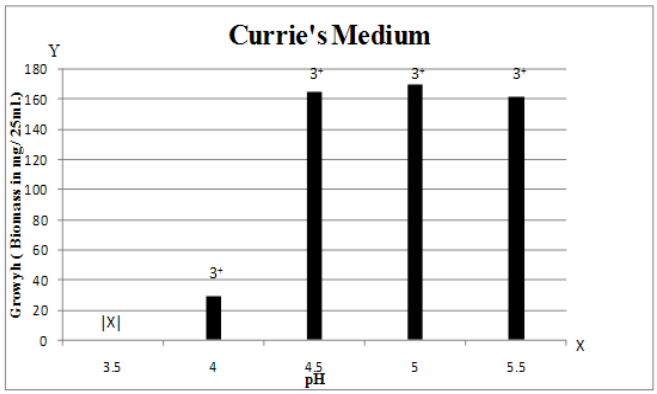

Fig. 3

Fig.: - 1, 2 & 3; Growth & sporulation of *Aspergillus umbrosus* in different mediums of different pH. Superscript on bar is degree of sporulation

Discussions & Conclusions

Braaksma *et. al.* (2009) experimented the influence of several environmental conditions on the production of extra cellular proteases of *A. niger*. They resulted that culture pH and nitrogen concentration particular strongly affected extra cellular proteases activities at pH 4 it was higher than at pH 5 and activities increase with increase in concentrations of ammonium as nitrogen source. Meena *et. al.* (2010) experimented the production of itaconic acid by different species of Aspergillus viz. *A. niger, A. terreus, A. nidulans* and *A. flavus*, in different pH. For commertial production they found that during fermentation medium with adjusted pH of 3.0, 3.5, 4.0, 5.0, 6.0 and 7.0. it was observed that the itaconic acid production was found to be maximum at pH 3.5 for all the selected fungal species. The levels of itaconic acid was found to increase with the pH from 3.0 to 3.5 and observed to decrease with further increase in pH from 3.5.The optimum pH for production was 3.5. They resulted that the level of itaconic acid the specific growth rate was maximum, the cell mass (Yx/s = 0.4976 g/g), yield factor is maximum for (Yp/x = 0.4387 g/g), the doubling time is minimum (14.84 hr.) for *A. terreus*. Astoreca *et. al.* (2010) studied the eco-physiology of *Aspergillus* section *nigri* species potential ochratoxin a producers on synthetic media & natural substrates. In all three mediums were experimented for *A. umbrosus*, growth were found nil at pH 3.5 in Asthana and Hawker's and Currie's medium, and in all cases it was optimum at pH 5.0, after that at pH 5.5 there were decrease in growth rate. Sporulation was moderate & secondary metabolite production in the form of reverse coloration was moderate i.e. light brown in Currie's medium at pH 5.0. Results indicated that amongst these three medium tested fungal growth, sporulation & secondary metabolite production is the best in Currie's medium but it was also not up to satisfactory level. Experiments regarding with all these three medium tested were not very satisfactory therefore some other medium were also taken for further studies.

A. umbrosus | *A. umbrosus* colonies in PDA (Potato Dextrose Agar) Medium | *A. umbrosus* (X320) in Currie's medium | *A. niger*

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